

Chapter 5

Acquiring Data

5.1 Starting an Acquisition

There are two ways of starting an acquisition:

- A single sample acquisition from the Tune window (Section 5.1.1).
- A multiple sample acquisition from the MassLynx window (Section 5.1.2).

5.1.1 Starting an Acquisition from the Tune Window

The easiest way to acquire data is directly from the Tune window:

- Acquisitions can be started and stopped.
- Most of the scanning parameters can be controlled.

However, when using this method:

- Inlet programs cannot be used.
- SIR and MRM data cannot be acquired.
- Analog data cannot be acquired.
- Multiple sample sequences cannot be acquired.

To start a single sample acquisition:

1. In the Tune window, click Acquire, or select Window > Acquire to open the Acquisition Setup dialog box (Figure 5-1).
2. Enter the parameters required to accommodate the required mass range and scan times (see Table 5-1 for details).
3. Click Start.

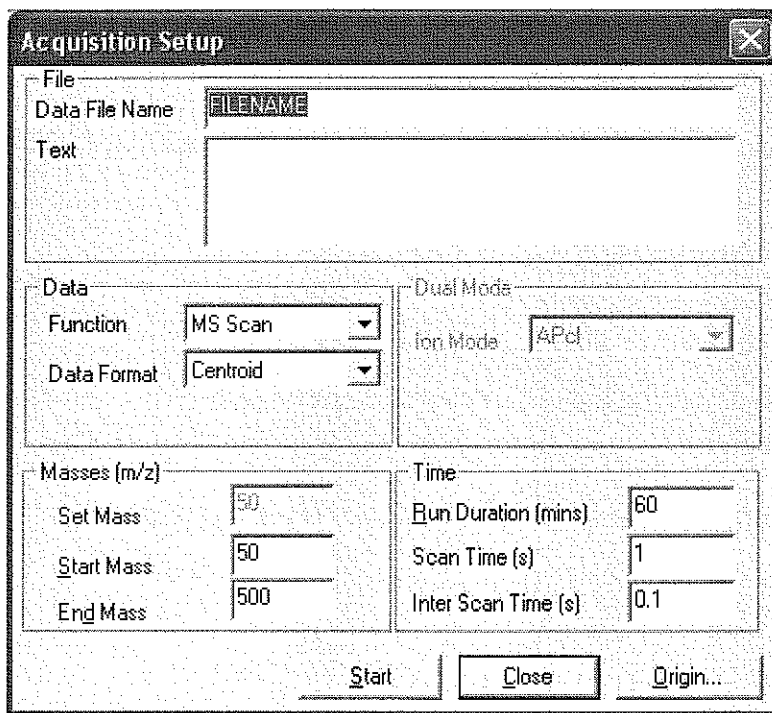


Figure 5-1 Acquisition Setup Dialog Box

Table 5-1 Acquisition Setup Dialog Box Parameters

| Parameter | Description |
|----------------|---|
| File | |
| Data File Name | The Data File Name can include up to 128 characters. If the file already exists on disk, you are prompted to rename the file or overwrite the existing one. The file is written to the data directory of the current project. |
| Text | The sample description, which can include up to 74 characters. The description can be displayed on any output of the acquired data. To display text on multiple lines, press Ctrl+Return at the end of a line. |

Table 5-1 Acquisition Setup Dialog Box Parameters (Continued)

| Parameter | Description |
|---------------------|--|
| Data | |
| Function | <p>The acquisition function used to collect the data can be any of:</p> <ul style="list-style-type: none"> • MS Scan • MS2 Scan • Daughter Scan • Parent Scan • Neutral Loss Scan • Neutral Gain Scan <p>See Section 5.7 for more information.</p> |
| Data Format | <p>The data format stored on disk can be any of:</p> <ul style="list-style-type: none"> • Centroid • Continuum • MCA <p>See "Types of Data Acquisition" on page 80 for more information.</p> |
| Masses (m/z) | |
| Set Mass | <p>Set Mass specifies the mass (Daughter Mass, Parent Mass, etc.) used for the particular function type. This control is disabled if the selected function does not require a set mass.</p> |
| Start Mass | <p>The mass (m/z) at which the scan starts.</p> |
| End Mass | <p>The mass (m/z) at which the scan stops.</p> <p>Note: <i>Start Mass must be lower than End Mass.</i></p> |
| Time | |
| Run Duration (mins) | <p>The length of the acquisition, in minutes.</p> |
| Scan Time (s) | <p>The duration of each scan, in seconds.</p> |
| Inter Scan Time (s) | <p>The time, in seconds, between a scan finishing and the next one starting. During this period no data are stored.</p> |

Table 5-1 Acquisition Setup Dialog Box Parameters (Continued)

| Parameter | Description |
|-----------|---|
| Origin | <p>Opens the Sample Origin dialog box. This allows you to specify additional information about the sample in the following fields:</p> <ul style="list-style-type: none"> • Submitter • Job • Task • Conditions |


To change the project into which data are saved:

1. Cancel the acquisition.
2. In the MassLynx window, select File > Open Project to open an existing project, or File > Project Wizard to create a new project.


5.1.2 Starting Multiple-Sample Acquisition from the MassLynx Window

The MassLynx window contains a Sample List for defining multiple samples which may be used together to perform a quantitative analysis. The list of samples is created using a spreadsheet style editor, which you can tailor to suit individual requirements. See the *MassLynx User's Guide* for more detailed information about multiple-sample Sample List analysis.

Procedure

1. Create a Sample List in the MassLynx window.
2. Select Run > Start, or click  to open the Start Sample List Run dialog box (Figure 5-2).
3. Enter the appropriate parameters (see Table 5-2 for details).
4. Click OK.
5. Repeat steps 1 to 4 as required.

Sample Lists are added to a queue and run sequentially unless Priority or Night Time Process has been selected.

Note:  in the Sample List denotes the sample currently being acquired.

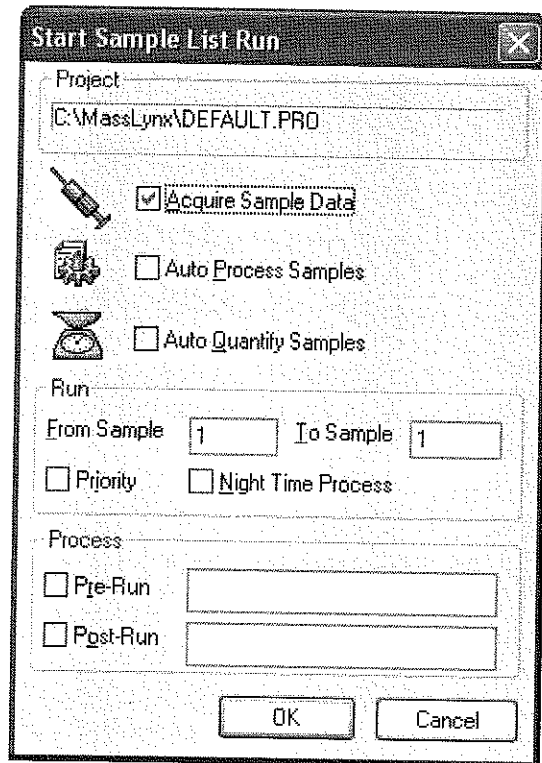


Figure 5-2 Start Sample List Run Dialog Box

Table 5-2 Start Sample List Run Dialog Box Parameters

| Parameter | Description |
|---|---|
| Project | The name of the current project. To send acquired data to a different project, exit this dialog box, open the other project, and restart the acquisition. |
| Acquire Sample Data | Acquires data from all the samples in the list. |
| Auto Process Samples | Processes acquired data as specified in the Sample List Process column. This may be existing data, or data newly acquired when the Acquire Sample Data option has been selected (see the <i>MassLynx User's Guide</i> for further details). |
| Auto Quantify Samples | Quantifies the acquired data using the method specified in the Quantify Samples dialog box (see Section 5.1.3). The current method will be used if a method is not specified in the Quantify Samples dialog box. If this option is selected, the Quantify Samples dialog box will open when the Start Sample List Run dialog OK button is clicked (see the <i>MassLynx User's Guide</i> for further details). |
| Note: The above three actions can run together or independently; i.e., the User can acquire, process, and quantify data simultaneously, or acquire data in one run and process or quantify it later. | |
| Run | |
| From Sample | The sample number of the first sample to be acquired. |
| To Sample | The sample number of the last sample to be acquired. |
| Priority | Marks a job as a priority process; it will be placed at the top of the queue, to run after a currently running job. |
| Night Time Process | Marks this entry as a night-time process. This option is useful for time-consuming acquisitions that would interrupt work on smaller acquisitions during the day. |
| Process | |
| Pre-Run | Runs the external executable process specified in the adjacent text box, which performs pre-processing on the samples. |
| Post-Run | Runs the external executable process specified in the adjacent text box, which performs post-processing on the samples. |

5.1.3 Automatic Quantification of the Sample List

The Quantify Samples dialog box (Figure 5-3) allows automatic processing of data files following acquisition. See the *MassLynx User's Guide* for more detailed information about using automated Sample List analysis.

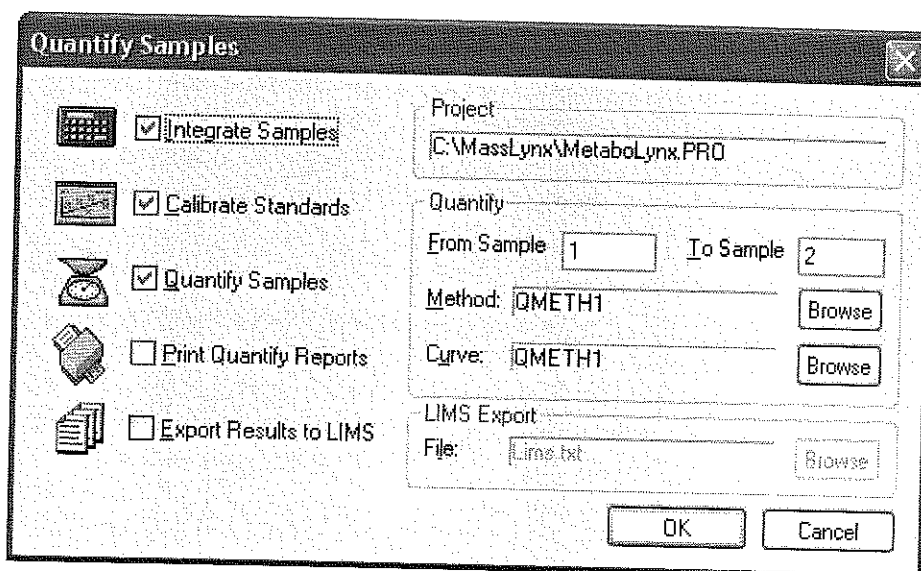


Figure 5-3 Quantify Samples Dialog Box

Procedure

1. Click Process Samples on the MassLynx Quantify shortcut bar, to open the Quantify Samples dialog box.
2. Enter the appropriate parameters (see Table 5-3 for details).
3. Click OK.

Table 5-3 Quantify Samples Dialog Box Parameters

| Parameter | Description |
|------------------------|--|
| Integrate Samples | Integrates all the sample data files named in the Sample List. |
| Calibrate Standards | Uses integration results to create and quantify calibration curves. <i>Note: Do not select this option if an existing calibration is to be used; instead, use the Curve Browse button to select the required calibration file.</i> |
| Quantify Samples | Uses integration results and quantify calibration curves to calculate compound concentrations. |
| Print Quantify Reports | Prints the results of integration and quantitation. |
| Export Results to LIMS | Produces a text file containing the quantitation results for use with a Laboratory Information Management System (LIMS). This option enables the LIMS Export pane File Browse button. Click Browse and select a file, or enter the name of a new one, then click Save. |
| Project | Displays the name of the current project. |
| Quantify | |
| From Sample: | The sample number of the first sample to be acquired. |
| To Sample: | The sample number of the last sample to be acquired. |
| Method | Displays the name of the current method file. To change the file, click the appropriate Browse button, and select a new file. |
| Curve | Displays the name of the current curve file. To change the file, click the appropriate Browse button and select a new file. |


5.2 Monitoring an Acquisition

5.2.1 Viewing the Status of an Acquisition

Acquisition status is shown in the MassLynx window. The run time is shown on the MS Status shortcut bar. The scan status, sample number, and scan number are shown on the status bar at the bottom of the window.

To view a scan-by-scan statistical report of the progress of an acquisition, select **Options > Acquisition Status** in the Tune window. This opens the Scan Report window, which displays details of the scan currently being acquired.

5.2.2 Viewing a Chromatogram in Real-Time

1. In the MassLynx window Sample List menu bar, click **Chromatogram** to open the Chromatogram window.
2. Click  or select **Display > Real-Time Update**. The chromatogram display is updated as the acquisition proceeds.

5.2.3 Viewing a Spectrum in Real-Time

1. In the MassLynx window Sample List menu bar, click **Spectrum** to open the Spectrum window.
2. Select **Display > Real-Time Update** to open the Spectrum Real-Time Update dialog box (Figure 5-4).

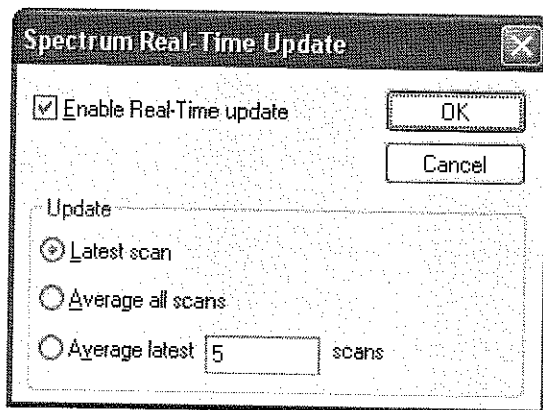



Figure 5-4 Spectrum Real-Time Update Dialog Box

3. Select Enable Real-Time update.
4. Select the required Update option (see the details below).
5. Click OK.

Note: Real-time updating can also be turned on and off by clicking .

When real-time update is enabled, the display is continually updated with spectra from the current acquisition. The actual information displayed is determined by clicking the appropriate update option:

- Latest scan displays the last acquired scan (the default option).
- Average all scans updates the display with spectra formed by averaging all the spectra acquired so far.
- Average latest scans updates the display with spectra formed by averaging the last n scans acquired, where n is specified in the associated text box.

5.3 Selecting Instrument Data Thresholds

Instrument data thresholding allows you to specify which data to acquire and store, and which to discard. Limiting the amount of data stored on disk can be useful when acquiring continuum data and performing long LC runs.

Instrument data threshold settings are specified in the Instrument Threshold Settings dialog box (Figure 5-5).

Changing Data Thresholding

1. In the Tune window, select Options > Set Instrument Threshold to open the Instrument Threshold Settings dialog box.
2. Make the required changes to the information. (The parameters are fully described in Sections 5.3.1 to 5.3.6.)
3. Click OK.

The new parameters are downloaded at the start of the next acquisition scan.

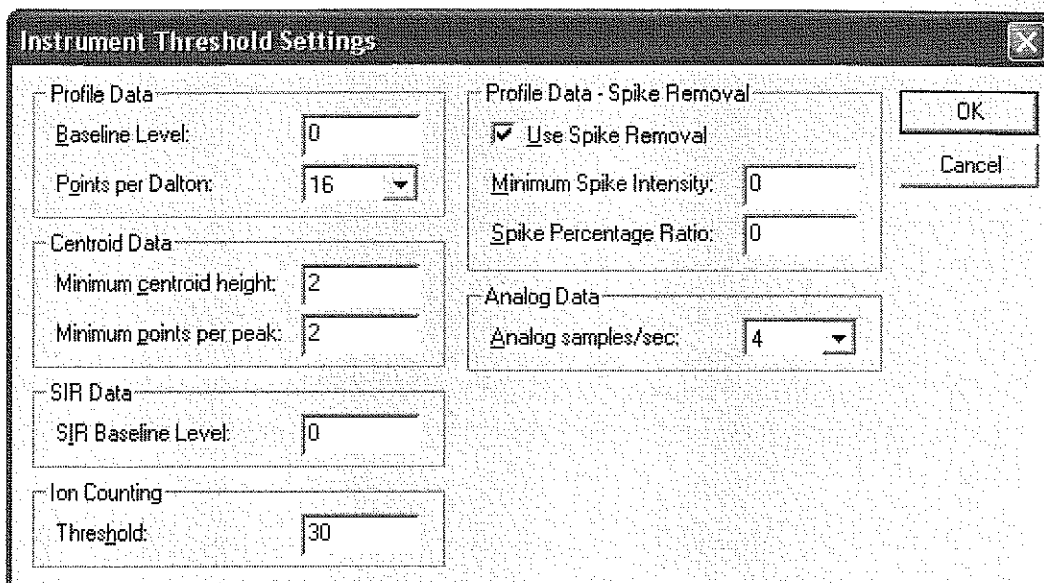


Figure 5-5 Instrument Threshold Settings Dialog Box

5.3.1 Profile Data

The Profile Data parameters control the amount of data collected during a continuum data acquisition.

Baseline Level changes the baseline position with respect to zero and is typically set to zero. A positive value increases the amount of noise seen. A negative value reduces the noise seen and acts as a form of thresholding applied to 1/16 Dalton-type samples.

The Baseline Level adjustment takes place after ion counting and, therefore, has a less significant effect than Ion Counting Threshold (see Section 5.3.4).

Points per Dalton can have one of three values: 4, 8, or 16:

- Acquiring data at 16 points per Dalton gives the greatest possible resolution.
- Selecting 8 points per Dalton instead of 16 produces data files approximately half as big.
- Acquiring data at 4 points per Dalton gives data with a smoothed appearance.

5.3.2 Centroid Data

Minimum centroid height sets a height below which detected peaks are ignored. This reduces the size of acquired data files and is useful when concentrating on larger peaks of interest. A suitable value can be deduced by evaluating spectral noise levels, and should be evaluated for each system.

The Minimum points per peak value specifies the minimum number of points that a continuum peak must comprise to initiate centroid processing. A typical value is 10.

5.3.3 SIR Data

The SIR Baseline Level value specifies the position of the SIR baseline above zero; it is typically set to zero. Increasing the value causes the baseline to appear higher in the spectrum.

5.3.4 Ion Counting Threshold

Ion Counting Threshold sets the intensity level below which a data point is ignored. This threshold is applied to all acquisitions, regardless of scanning mode. It is also the most significant data thresholding parameter, as it is the first to be applied to raw data.

When an acquisition is started, the instrument performs a prescan with the ion beam switched off. This allows measuring of the acquisition system electronic noise level and its standard deviation. Ion Counting Threshold affects only the system electronic noise level.

The specified Ion Counting Threshold level is multiplied by 1/10 of the standard deviation of the noise to determine the suitable intensity level. Hence a value of 10 equates to one standard deviation of the electronic noise level.

- Values can be set between 0 and 1000; the higher the number, the more data is discarded.
- If a value of 0 is entered, the intensity level is set so that it sits in the middle of the noise. This means that roughly half the noise data is acquired.
- A value of 10 places the threshold just above the noise, so almost all the noise data is acquired.
- If a value of 200 is specified, the threshold sits well above the noise level, so little noise data is acquired.
- A value of 30 is suitable for most data.

Note: *Ion Counting Threshold should be set to 0 when acquiring data to be analyzed using MaxEnt™, as the MaxEnt algorithm needs to measure noise accurately within a data file (see the MassLynx User's Guide for full details).*

Set Ion Counting Threshold so that background noise is removed without significantly reducing the intensity of the smallest peaks of interest.

5.3.5 Profile Data – Spike Removal

Spikes can be distinguished from real data because the peaks are very narrow and, when compared to their immediate neighbors, very intense. A data point determined to be a spike is removed by setting the value of the data point to the average of its immediate neighbors.

Spike removal involves additional processing during data acquisition and so reduces the maximum achievable acquisition rates by approximately 30%.

Performing Spike Removal During an Acquisition

1. In the Instrument Threshold Settings dialog box Profile Data - Spike Removal pane, select Use Spike Removal.
2. Examine the peak intensities displayed in the Tune window to deduce a suitable value for the intensity threshold below which spikes are ignored. Set Minimum Spike Intensity to this value.

A very low intensity signal may include single ion events that can be combined to produce significant peaks. For this type of data, set Minimum Spike Intensity to a suitable value such that these single ion events are not discarded as spikes.

3. Set a suitable value for Spike Percentage Ratio.

This ratio determines whether a data point is a spike by comparing the data point to its immediate neighbors. For example, with Spike Percentage Ratio set to 33%, a data point is regarded as a spike if its intensity is three times (or more) greater than both its immediate neighbors. A setting of 20% requires an intensity ratio of 5:1 to identify a spike.

4. Click OK to accept any changes.

5.3.6 Analog Data


Select the number of samples to acquire, per second, from the Analog samples/sec drop-down list.

5.4 Checking the Status of the Communications Link


To check the status of the communications link between the MassLynx PC and embedded PC, select Options > Communications Status, in the Tune window, to open the System Manager window. This displays details of the embedded system's status.

5.5 Stopping an Acquisition

You can stop an acquisition from either the Tune window or the MassLynx window. Data already acquired are saved.

- In the Tune window, click .



Note: Stopping an acquisition from the Tune window will stop only the current acquisition in the Sample List; data acquisition for the next entry in the Sample List will then start.

- In the MassLynx window, select Run > Stop, or click .

Note: Stopping an acquisition from the MassLynx window will pause the Sample List.

5.6 Pausing an Acquisition

You can pause an acquisition from either the Tune window or the MassLynx window:

- In the Tune window, click .
- In the MassLynx window, select Run > Pause or click .

Click the appropriate pause button again to restart the acquisition.

5.7 Setting-Up Scanning Functions

5.7.1 Creating a Function List

A function list contains the function(s) used by the mass spectrometer to scan the instrument during an acquisition; it is created using the MS Method Editor (Figure 5-6). A Function List can be a mixture of different scanning techniques that run either sequentially or concurrently during an acquisition.

Click the MS Method icon in the MassLynx window to open the MS Method Editor.

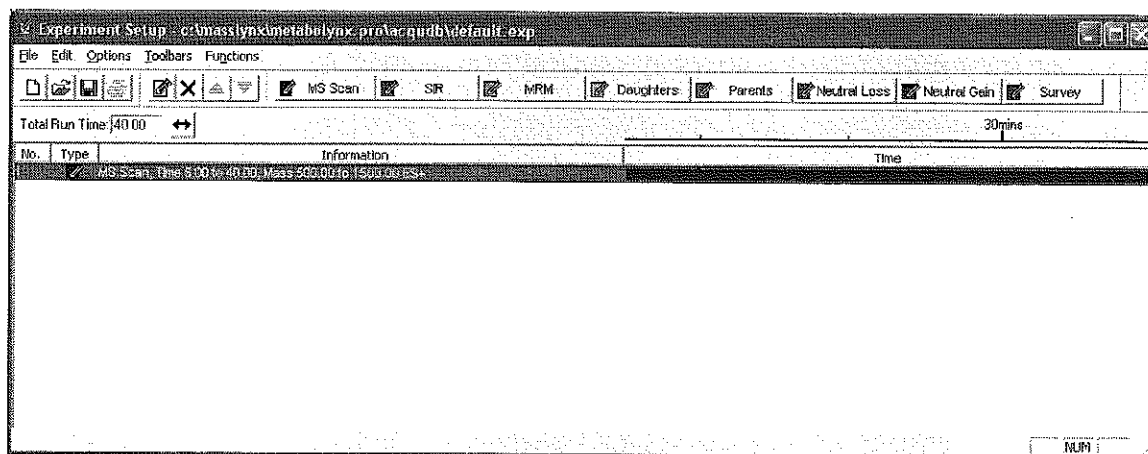


Figure 5-6 MS Method Editor

Mixed function acquisitions are typically used to acquire different SIR groups over different retention windows.

Once created, a Function List can be saved, and then reopened when an acquisition is started.

Figure 5-6 shows a simple Function List containing only one function: a full scan in centroid mode, between 500 and 1500 Da using ES+ ionization. Immediately above the function bar display is a time scale, which shows the elapsed time at which the function becomes active and for how long it runs. In this case, the function starts after 5 minutes and then runs for 35 minutes, terminating after a total elapsed time of 40 minutes.

A more complicated Function List, with four SIR functions, each running sequentially for 5 minutes, is shown in Figure 5-7.

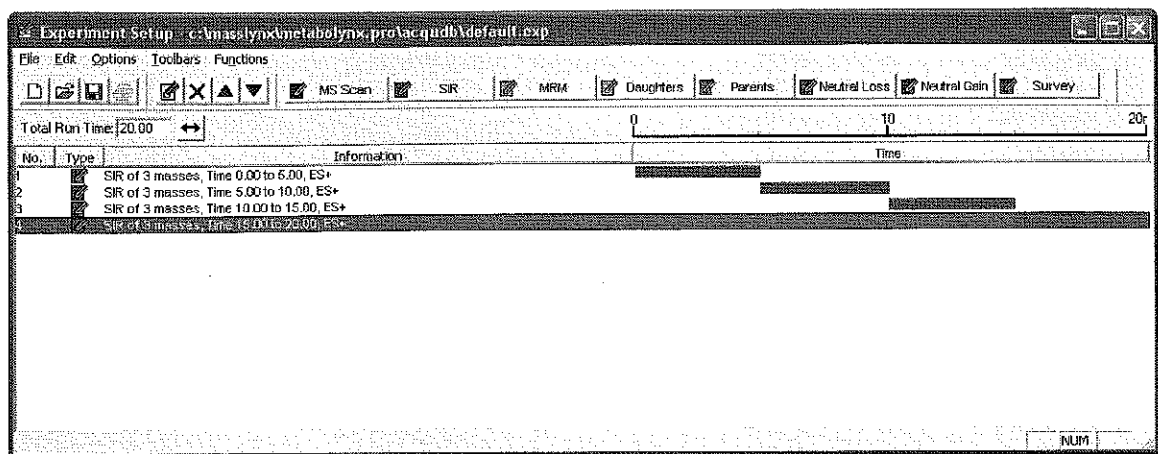


Figure 5-7 MS Method Editor with Four SIR Functions

The currently selected function is highlighted and enclosed in a rectangular frame. If the Function List contains several functions, a function can be selected by clicking on it or by using the keyboard arrow keys.

MS Method Editor Toolbar

The MS Method Editor toolbar functions are shown in Table 5-4.

Table 5-4 MS Method Editor Toolbar Buttons















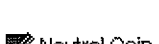

| Toolbar Button | Function |
|---|--|
|  | Create a new Function List. |
|  | Open an existing Function List. |
|  | Save the current Function List to disk. |
|  | Print the current window in portrait format. Note: To print the current window in landscape format, select File > Print. |
|  | Edit the selected function. |
|  | Delete the selected function. |

Table 5-4 MS Method Editor Toolbar Buttons (Continued)


| Toolbar Button | Function |
|---|---|
|  | Move the selected function up the list of functions. |
|  | Move the selected function down the list of functions. |
|  | Create a new Full Scan function (see Section 5.7.2). |
|  | Create a new SIR function (see Section 5.7.3). |
|  | Create a new MRM function (see Section 5.7.5). |
|  | Create a new Daughter function (see “Setting-Up a Daughter Scan” on page 85). |
|  | Create a new Parent function (see “Setting-Up a Parent Scan” on page 87). |
|  | Create a new Neutral Loss function (see “Setting-Up a Neutral Loss Scan” on page 87). |
|  | Create a new Neutral Gain function (see “Setting-Up a Neutral Gain Scan” on page 87). |
|  | Create a new Survey function (see Section 5.7.6). |

Adding a New Function to the List

1. Click one of the toolbar buttons, or choose the required function from the Functions menu to open the appropriate Function Editor, showing default values.
2. Make required changes to the parameters.
3. Click OK. The new function is added to the Function List.

Note: The Function Editors are described in Sections 5.7.2 to 5.7.5.

Modifying an Existing Function in the List

1. Select the function.
2. Click  or double-click on the function to open the appropriate Function Editor, showing default values.
3. Make the required changes to the parameters.
4. Click OK. The Function List display is updated to show changes.

Note: The Function Editors are described in Sections 5.7.2 to 5.7.5.

Copying an Existing Function in the List



1. Select the function.
2. Select Edit > Copy.
3. Select Edit > Paste. A copy of the function appears in the Function List.
4. Modify the parameters (see above).

Removing a Function from the List


1. Select the function.
2. Click , select Edit > Delete, or press the Del key.

Changing the Order of Functions in the List

Functions are displayed in ascending start and end time order; this order cannot be changed. For functions with identical start and end times, you can change the order in which they are performed:

1. Select the required function.
2. Repeatedly click  or  until the function is in the required position.

Setting the Maximum Retention Time

1. Enter the required value in the Total Run Time: text box.
2. Click . The ratio of the defined functions is maintained. For example, if two functions are defined, one from 0 to 5 minutes, and the other 5 to 10 minutes, then a Total Run Time of 10 minutes is displayed. If this value is changed to 20, the first function now runs from 0 to 10 minutes, and the second from 10 to 20 minutes.

Setting Solvent Delays

No data is stored during a solvent delay period, which means that solvent peaks that would normally be seen eluting on the Total Ion Current (TIC) chromatogram are not seen.

Select Options > Solvent Delay on the MS Method Editor to open the Solvent Delay dialog box (Figure 5-8). Up to four solvent delay periods can be entered in the dialog box.

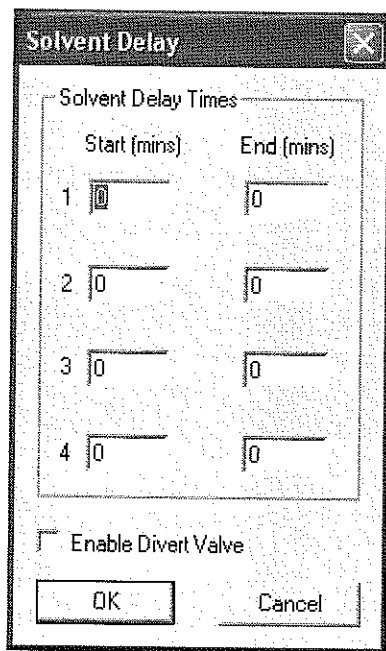


Figure 5-8 Solvent Delay Dialog Box

For APCI functions, the APCI probe temperature is set to the value specified on the Tune window APCI+ Source page, APcI Probe Temp field for each solvent delay period.

Select Enable Divert Valve to use the divert/injector valve in the divert mode. This diverts the flow of solvent during a solvent delay period either to, or away from, the source for the time period.

Acquiring Analog Data

Up to eight channels of analog data can be acquired. These are stored with the data acquired from the mass spectrometer.

Analog channels are typically used to collect data from external units such as UV detectors, which must be connected to the Analogue Channels connectors (see Section 1.6).

Note: Although eight channel inputs are available, the MassLynx software can only control four of them (see below).

A reading is made from the external channel at the end of each scan and stored with the data for that scan. The resolution of the chromatography for an analog channel therefore depends on the scan speed used to acquire the mass spectrometry data.

To store data for an analog channel:

1. On the MS Method Editor, select Options > Analog Data to open the Analog Data dialog box (Figure 5-9).

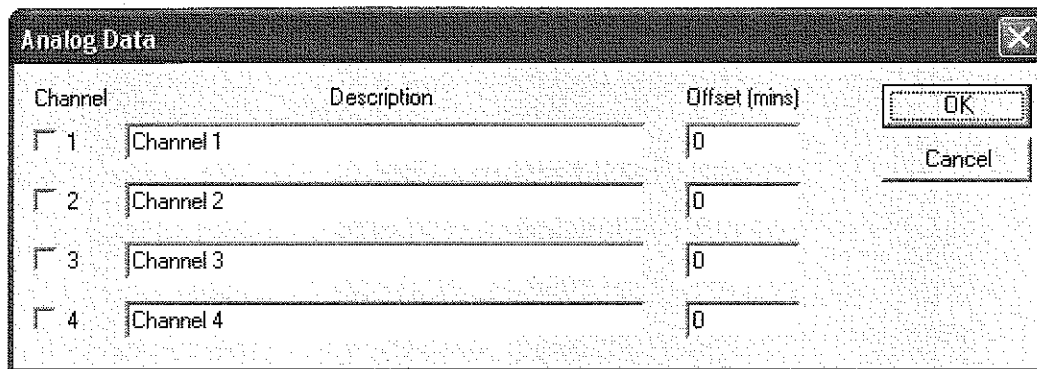


Figure 5-9 Analog Data Dialog Box

2. Select the box(es) for the required analog channel(s).
3. Enter a textual description for each selected channel.

This description is used on the analog Chromatogram dialog box as the channel description (see the *MassLynx User's Guide*).

4. Enter an Offset (mins) to align the external unit with the mass spectrometer.
5. Click OK.

Saving a Function List

1. On the MS Method Editor, select File > Save As to open a standard Save As dialog box.
2. Enter a new file name, or select an existing file from the displayed list.
3. Click Save.

Opening a Function List

1. On the MS Method Editor, select File > Open to open a standard Open dialog box.
2. Enter the required file name, or select an existing file from the displayed list.
3. Click Open.

5.7.2 Setting-Up a Full Scan Function

The full scan Function Editor (Figure 5-10) is used to set-up centroid, continuum, and MCA functions.

Function: 2 MS Scan

Mass (m/z)

Start: 50

End: 600

Time (Mins)

Start: 0

End: 60

Cone Voltage

Use Tune Page

Cone Voltage (V): 30

Use Cone Voltage Ramp

CV Ramp...

Method

Ionization Mode: ESI

Data: Centroid

Scan Duration (secs)

Scan Time: 1

Inter-Scan Delay: 0.1

APCI Probe

Use Tune Page Settings

Probe Temp: 100

OK Cancel

Figure 5-10 Full Scan Function Editor


1. On the MS Method Editor, click  MS Scan, or select Functions > MS Scan to open the full scan Function Editor.
2. Enter the required parameters (see Table 5-5 for details).

Table 5-5 Full Scan Function Editor Parameters

| Parameter | Description |
|-----------------------|---|
| Mass (m/z) | |
| Start | The mass (m/z) at which the scan starts. |
| End | The mass (m/z) at which the scan stops. Note: <i>The Start mass must be less than the End mass.</i> |
| Time (Mins) | |
| Start | The retention time (in minutes) at which the scan starts. |
| End | The retention time (in minutes) at which the scan stops. |
| Cone Voltage | |
| Use Tune Page | When selected, the cone voltage set in the Tune window at the start of the acquisition is used. Note: <i>The cone voltage value cannot be altered during acquisition by typing new values into the Tune window, since the new values are not downloaded during acquisition.</i> |
| Cone Voltage (V) | The cone voltage. |
| Use Cone Voltage Ramp | When selected, applies a ramp to linearly increase the cone voltage (see "Applying a Linear Ramp to the Cone Voltage" on page 79). |
| CV Ramp | Opens the Cone Ramp dialog box (see "Applying a Linear Ramp to the Cone Voltage" on page 79). Note: <i>CV Ramp is available only when Use Cone Voltage Ramp is selected.</i> |
| Method | |
| Ionization Mode | Specifies the ionization mode and polarity applied during data acquisition. |
| Data | Specifies the type of data collected and stored on disk (see "Types of Data Acquisition" on page 80). |
| Scan Duration (secs) | |
| Scan Time | The duration of each scan in seconds. |
| Inter-Scan Delay | The time between a scan's end and the next one's start, in seconds. No data are stored during this period. |

Table 5-5 Full Scan Function Editor Parameters (Continued)

| Parameter | Description |
|------------------------|--|
| APCI Probe | Note: The parameters in this pane are enabled when Ionization Mode is set to API. |
| Use Tune Page Settings | When selected, the APCI probe temperature set in the Tune window at the start of the acquisition is used. Note: The APCI probe temperature value cannot be altered by typing new values into the Tune window during the acquisition, since the new values are not downloaded during the acquisition. |
| Probe Temp | The probe temperature, in degrees centigrade. |

Applying a Linear Ramp to the Cone Voltage

1. On the Full Scan Function Editor, select Use Cone Voltage Ramp.
2. Click CV Ramp to open the Cone Ramp dialog box (Figure 5-11).

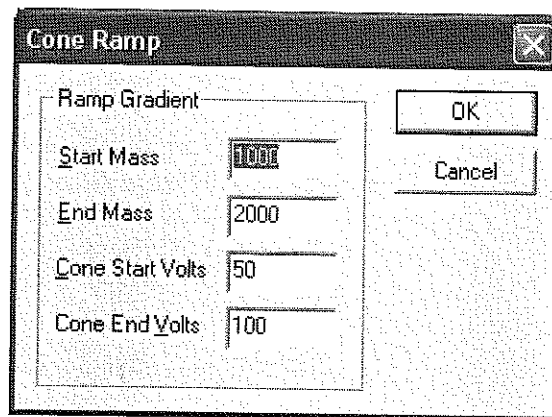


Figure 5-11 Cone Ramp Dialog Box

3. Enter the required parameters (see Table 5-6 for details).

Table 5-6 Cone Ramp Dialog Box Parameters

| Parameter | Description |
|---------------|--|
| Ramp Gradient | |
| Start Mass | The mass (m/z) at which the lower cone voltage is specified. |

Table 5-6 Cone Ramp Dialog Box Parameters (Continued)

| Parameter | Description |
|------------------|--|
| End Mass | The mass (m/z) at which the upper cone voltage is specified. |
| Cone Start Volts | The lower cone voltage. |
| Cone End Volts | The upper cone voltage. |

Note: The two values of cone voltage specify a cone voltage gradient that is then extrapolated to cover the full mass range.

Types of Data Acquisition

In the Full Scan Function Editor (see Figure 5-10), the Data parameter specifies the type of data to be collected and stored on disk:

- Centroid – Stores data as centroid, intensity, and mass-assigned peaks. Data are stored for every scan.
- Continuum – The signal received by the interface electronics is stored regularly to give an analog intensity picture of the data being acquired. Data are not processed into centroid peaks, but are stored for every scan.

As data are always acquired and stored, even when no peaks are acquired, data files tend to be significantly larger than those for centroid data. It is possible, however, to set a threshold below which the data are not stored. Depending on the nature of the data acquired, this can greatly reduce these effects. The threshold can be set so that data considered to be “noise” can be discarded, thus improving data acquisition speed and reducing data file sizes. For more information about setting data thresholds, see Section 5.3.

- MCA (Multi Channel Analysis) – MCA data can be thought of as “summed continuum”, with only one intensity-accumulated scan stored for a given experiment. As each scan is acquired, its intensity data is added to the accumulated, summed data of previous scans.

An advantage of MCA is that random noise does not accumulate as rapidly as real data and therefore effectively averages out over a number of scans. This emphasizes the real data and improves the signal-to-noise ratio.

Another advantage of MCA is that scanning speeds can be increased and significantly less storage space is required, because data is stored only at the end of an experiment.

The disadvantage of MCA is that, because there is only one scan, it cannot be used for time-resolved data.

5.7.3 Setting-Up an SIR Function

The SIR (Selected Ion Recording) technique is typically used when only a few specific masses are to be monitored. Since most of the data acquisition time is spent on these masses, the technique is far more sensitive than full scanning.

The SIR Function Editor (Figure 5-12) is used to specify the masses to be monitored, along with their dwell times, spans, and inter-channel delay times.

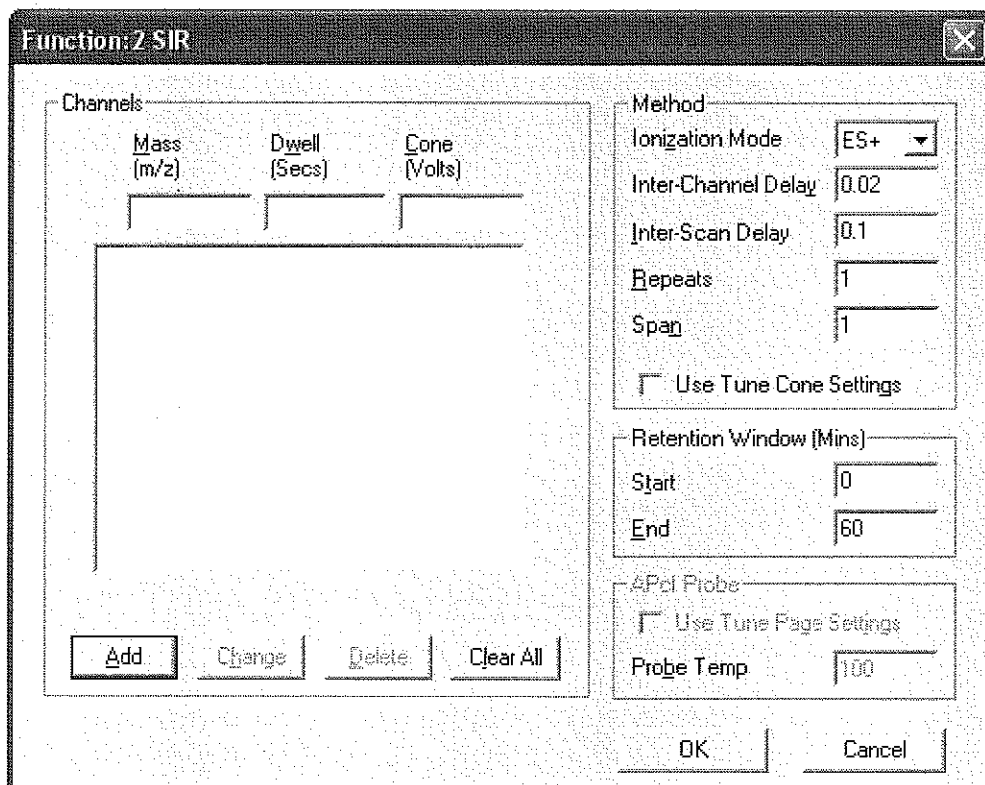


Figure 5-12 SIR Function Editor

Procedure


1. On the MS Method Editor, click  SIR, or select Functions > MS SIR to open the SIR Function Editor.
2. Enter the required parameters (see Table 5-7 for details).

Table 5-7 SIR Function Editor Parameters

| Parameter | Description |
|------------------------|--|
| Channels | |
| Mass (m/z) | The mass (m/z) to be scanned. |
| Dwell (Secs) | The length of time, in seconds, for which the highlighted mass is monitored. |
| Cone (Volts) | The cone voltage. |
| Add | Adds the current values in the Mass, Dwell, and Cone boxes to the Function List (see "Adding a Function to the SIR Function List" on page 83). |
| Change | Replaces the values in the selected function by the values currently in the Mass, Dwell, and Cone boxes (see "Modifying a Function in the SIR Function List" on page 83). |
| Delete | Deletes the selected function from the list (see "Deleting a Function from the SIR Function List" on page 83). |
| Clear All | Deletes all the functions from the list (see "Deleting All Functions from the SIR Function List" on page 83). |
| Method | |
| Ionization Mode | The ionization mode and polarity used during data acquisition. |
| Inter-Channel Delay | The time, in seconds, between finishing monitoring the current mass and starting monitoring the next mass in the Function List. |
| Inter-Scan Delay | The time, in seconds, between finishing one scan and starting the next scan. |
| Repeats | The number of repeats of the function. Repeats is only relevant for experiments having more than one function. |
| Span | Specifies a small mass window applied centrally about the current mass. During acquisition, this range is scanned over the specified Dwell time. A span of zero can be set to scan the specified mass without a mass window. |
| Use Tune Cone Settings | When selected, the cone voltage set in the Tune window at the start of the acquisition is used. <i>Note: The cone voltage value cannot be altered during acquisition by typing new values into the Tune window, since the new values are not downloaded during acquisition.</i> |

Table 5-7 SIR Function Editor Parameters (Continued)

| Parameter | Description |
|-------------------------|--|
| Retention Window (Mins) | |
| Start | The start of the retention time, in minutes, during which the current function is active. |
| End | The end of the retention time, in minutes, during which the current function is active. |
| APCI Probe | Note: The parameters in this frame are enabled when Ionization Mode is set to API. |
| Use Tune Page Settings | When selected, the APCI probe temperature set in the Tune window at the start of the acquisition is used. Note: The APCI probe temperature value cannot be altered by typing new values into the Tune window during the acquisition, since the new values are not downloaded during the acquisition. |
| Probe Temp | The probe temperature, in degrees centigrade. |

Adding a Function to the SIR Function List

Up to 32 functions can be entered in the Function List.

1. Enter the required values into the Mass, Dwell, and Cone boxes.
2. Click Add.

Modifying a Function in the SIR Function List

1. Click on the function to select it.
The values for the selected function are displayed in the Mass, Dwell, and Cone boxes.
2. Change the values in the Mass, Dwell, or Cone boxes as required.
3. Click Change to update the values in the Function List.

Deleting a Function from the SIR Function List

1. Click on the function to select it.
2. Click Delete.

Deleting All Functions from the SIR Function List

To delete all the functions from the Function List, click Clear All.

5.7.4 Setting-Up MS/MS Scanning Functions

1. On the MS Method Editor (see Figure 5-6), click the appropriate toolbar function button to open the appropriate Function Editor. The MS/MS scanning Function Editors are shown in Figure 5-13.

Note: No toolbar button exists for the MS2 scan function. Select *Functions > MS2 Scan* to open the MS2 Function Editor.

2. Enter the required parameters (see the following sections).

Note: Many of the fields in the MS/MS editors are similar to those in the Full Scan Function Editor. Only fields that differ significantly are described.

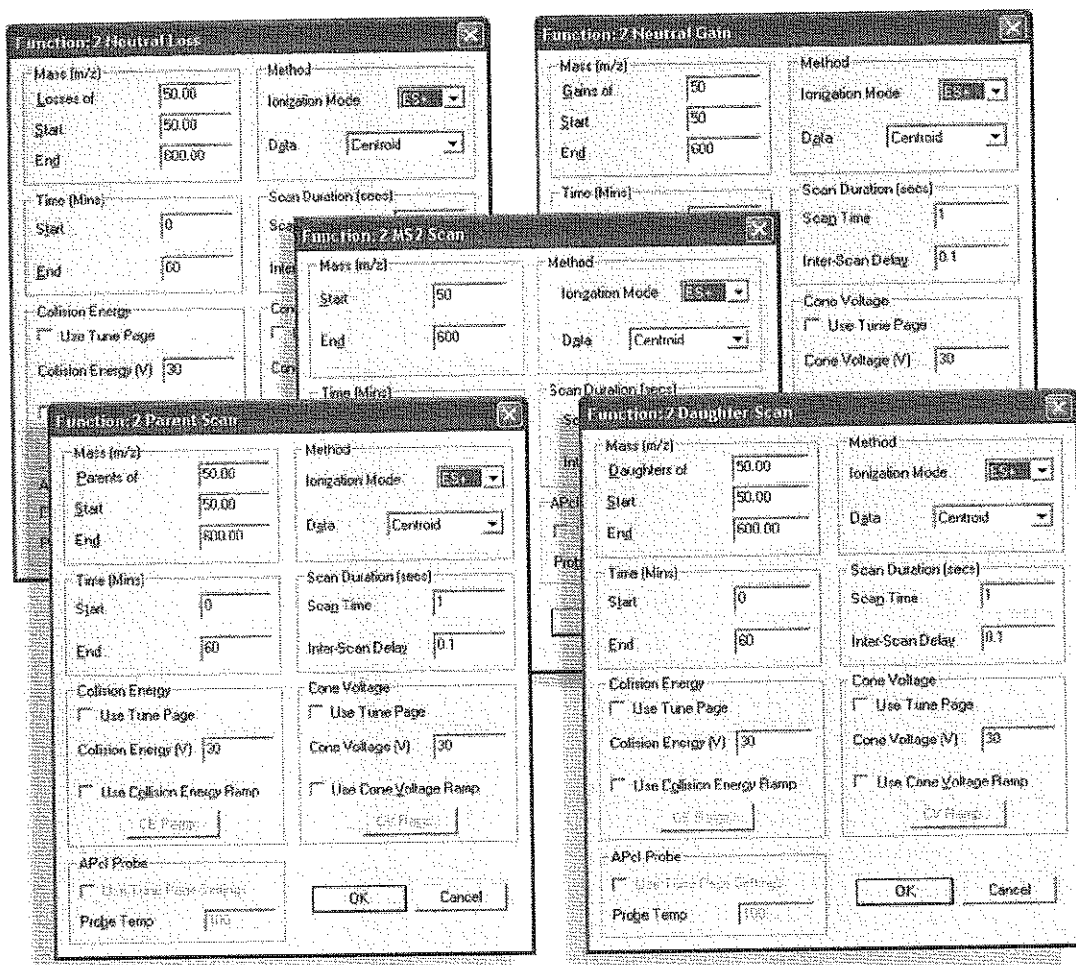


Figure 5-13 MS/MS Scanning Function Editors

Setting-Up a Daughter Scan

This is the most commonly used MS/MS mode; it is used to look at fragmentations of a particular ion. MS1 is set to the parent (precursor) mass and is not scanned.

The resolution of MS1 can be lowered until the peak width at the base is two masses wide, without the daughter spectrum containing any ions from the adjacent parent masses.

You may specify a daughter mass greater than the parent (precursor) mass. In this case, ions which have gained mass in the collision cell, or are of higher mass-to-charge ratio (for example, when a multiply-charged ion fragments and loses a charge) are detected.

Table 5-8 describes the Daughter Scan Function Editor parameters.

Table 5-8 Daughter Scan Function Editor Parameters

| Parameter | Description |
|---------------------------|--|
| Mass (m/z) | |
| Daughters of | The parent mass (m/z). |
| Start | The mass (m/z) at which the MS2 scan starts. |
| End | The mass (m/z) at which the MS2 scan ends. |
| Collision Energy | |
| Use Tune Page | When selected, the collision energy set in the Tune window at the start of the acquisition is used. <i>Note: The collision energy value cannot be altered during acquisition by typing new values into the Tune window, since the new values are not downloaded during acquisition.</i> |
| Collision Energy (V) | The collision energy, in electron volts, to be used for the collision cell during the scan. |
| Use Collision Energy Ramp | When selected, applies a linear ramp to the collision energy (see "Applying a Linear Ramp to the Collision Energy" on page 86). |
| CE Ramp | Opens the Collision Ramp dialog box (see "Applying a Linear Ramp to the Collision Energy" on page 86). <i>Note: CE Ramp is available only when Use Collision Energy Ramp is selected.</i> |

Applying a Linear Ramp to the Collision Energy

1. On the Function Editor, select Use Collision Energy Ramp.
2. Click CE Ramp to open the Collision Ramp dialog box (Figure 5-14).

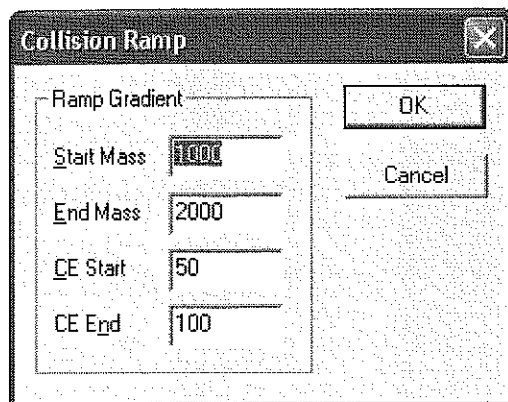


Figure 5-14 Collision Ramp Dialog Box

3. Enter the required parameters (see Table 5-9 for details).

Table 5-9 Collision Ramp Dialog Box Parameters

| Parameter | Description |
|---------------|--|
| Ramp Gradient | |
| Start Mass | The mass (m/z) at which the lower collision energy is specified. |
| End Mass | The mass (m/z) at which the upper collision energy is specified. |
| CE Start | The lower collision energy. |
| CE End | The upper collision energy. |

Note: The two values of collision energy specify a collision energy gradient that is then extrapolated to cover the full mass range.

Setting-Up a Parent Scan

This mode is used to look for the parent of a particular fragment.

MS2 is set to the mass of the fragment and is not scanned.

There are often several masses from which a daughter may come, so that any one fragment is derived from a number of different peaks.

Table 5-10 describes the parameters unique to the Parent Scan Function Editor.

Table 5-10 Parent Scan Function Editor Parameters

| Parameter | Description |
|---|--|
| Mass (m/z) | |
| Parents of | The daughter mass (m/z). |
| Start | The mass (m/z) at which the MS1 scan starts. |
| End | The mass (m/z) at which the MS1 scan ends. |
| <i>Note: Start is normally set just below Parents of and End to a value above the highest expected parent mass.</i> | |

Setting-Up an MS2 Scan

In this mode, MS2 is resolving while MS1 transmits ions over a wide mass range. While this scanning mode can be used for acquiring data, it is mostly used in the Tune window for setting and optimizing the acquisition conditions.

Setting-Up a Neutral Loss Scan

In this mode, the peak in a spectrum that gives the neutral loss specified in Losses of is detected. The precursor mass is scanned in MS1 and MS2 is scanned at this mass less the neutral loss mass. Starting masses are therefore detected on the mass scale of MS1. Start (for MS1) should be greater than Losses of to give MS2 a valid start mass.

Setting-Up a Neutral Gain Scan

This is an infrequently used mode, since the mass selected by MS2 is seldom higher than that of MS1. It applies to studies where a precursor ion gains mass by ion molecule reaction or where multiply-charged ions fragment into particles with higher m/z values.

5.7.5 Setting-Up an MRM Function

Multiple reaction monitoring (MRM) functions are set-up in a similar way to SIR functions (see Section 5.7.3) but allow a number of MS/MS transitions (fragmentations) between MS1 and MS2 to be monitored.


All fields in the MRM Function Editor (Figure 5-15) are similar to those described in Section 5.7.3.

| Parent (m/z) | Daughter (m/z) | Dwell (Secs) | Cone (Volts) | Coll Energy (eV) |
|--------------|----------------|--------------|--------------|------------------|
| 455.2 | 165.2 | 0.1 | 50 | 30 |

Figure 5-15 MRM Function Editor

5.7.6 Setting-Up a Survey Function

Survey scans are used to search for precursor ions.

1. In the MS Method Editor (see Figure 5-6), delete any existing functions from the Function List.
2. Click  Survey or select Functions > Survey Scan to open the Survey Scan Function Editor.

Note: The MS Method Editor does not add survey functions to the list if non-survey functions are present.

3. Select the required tab and specify the appropriate parameters (see below).

Setting-Up the Parameters for MS and MS/MS Scanning

The Survey and MSMS Template pages (Figure 5-16) allow you to specify the parameters for MS and MS/MS scanning during the survey; they are similar to the MS/MS scanning Function Editor pages (see Section 5.7.4). The MSMS Template page also allows you to select the required Instrument Parameter file (.ipr).

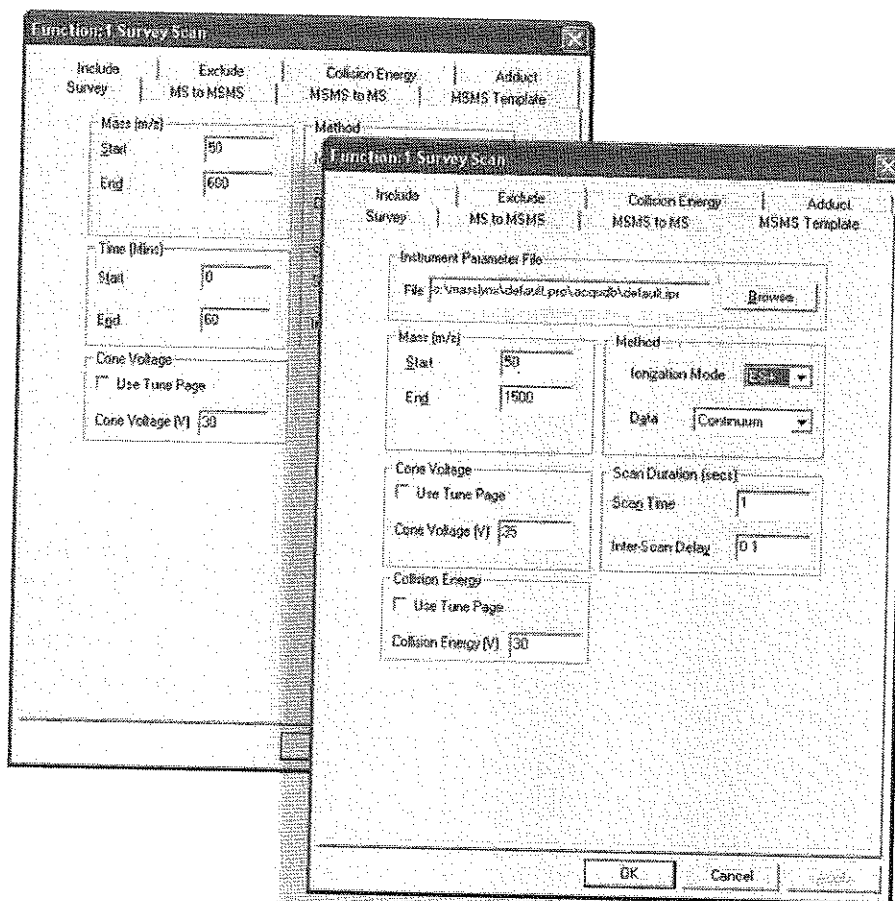


Figure 5-16 Survey Scan Function Editor: Survey and MSMS Template Pages

Setting-Up MS to MS/MS Switching

The MS to MSMS page (Figure 5-17) allows MS to MS/MS switching to be set-up.

Function:1 Survey Scan

Include Survey | Exclude MS to MSMS | Collision Energy MSMS to MS | Adduct MSMS Template

MS to MSMS Switch Criteria

TIC

Intensity

Threshold: 10

Detection Window (Da): 0.5

Number of Components: 1

Retention Time Window (s): 1

Charge State

Tolerance Window +/- (Da): 3

Extraction Window (Da): 2

Precursor Selection

Everything

Included Masses only

Included Masses Take Priority

Detected Precursor Inclusion

Auto Exclude

Always Include

Include After Time (s): 11

Data

Discard uninteresting survey scans

OK Cancel Apply

Figure 5-17 Survey Scan Function Editor: MS to MSMS Page

Table 5-11 describes the MS to MSMS page parameters.

Table 5-11 Survey Scan Function Editor: MS to MSMS Page Parameters

| Parameter | Description |
|----------------------------|---|
| MS to MSMS Switch | |
| Criteria | |
| TIC | When selected, the switch from MS to MS/MS scanning occurs when the TIC of the spectrum rises above the specified Threshold value. |
| Intensity | When selected, the switch from MS to MS/MS scanning occurs when the intensity of the largest peak rises above the specified Threshold value. |
| Threshold | The value at which the switch from MS to MS/MS scanning occurs (TIC or Intensity). |
| Detection Window (Da) | When a peak is detected, no other peaks are sought within the mass range specified by plus or minus this value, centered about the detected peak. |
| Number of Components | Selects the maximum number of peaks of interest to detect from an MS scan. The software will attempt to find the Number of Components from an MS spectrum. Once the maximum number of components in the spectrum for the specified criteria is found, new MS/MS functions are generated automatically and a switch to MS/MS scanning performed. |
| Retention Time Window (s) | The Retention Time Window is used by the Include and Exclude lists. A mass will be included or excluded if it appears at the specified retention time plus or minus this value. |
| Charge State | |
| Tolerance Window +/- (Da) | If a peak is detected, but it is outside the Tolerance Window of where it should be, it is ignored by the Charge State Recognition routines. |
| Extraction Window (Da) | The window around the base peak for data to be extracted, in Da. For example, if the Extraction Window is 1 Da, the window will extend 0.5 Da either side of the base peak. The extracted data is then used to calculate the charge state. |
| Precursor Selection | |
| Everything | Monitors all the valid masses satisfying the selection criteria. |

Table 5-11 Survey Scan Function Editor: MS to MSMS Page Parameters (Continued)

| Parameter | Description |
|------------------------------------|---|
| Included Masses only | Monitors only the masses in the Include List. |
| Included Masses Take Priority | Masses on the Include List are given priority. If no precursors are found, other valid masses are monitored. <i>Note: A mass is valid if it is not on the Exclude List (see "Editing the Exclude List" on page 101) and it satisfies the precursor selection criteria.</i> |
| Detected Precursor Inclusion | |
| Auto Exclude | Only switch on the masses once during an acquisition. |
| Always Include | Never exclude any masses from being switched on. |
| Include After Time (s) | Once a mass has been switched on, it will be excluded for the time specified in the adjacent text box. Once the retention time of a mass has exceeded this value, the mass can be considered as a peak of interest again. |
| Data | |
| Discard uninteresting survey scans | Stores only the survey scans that detect precursor ions. This saves on disk space because survey scans that contain no relevant data are rejected. |

Setting-Up MS/MS to MS Switching

The MSMS to MS page (Figure 5-18) allows MS/MS to MS switching to be set-up.

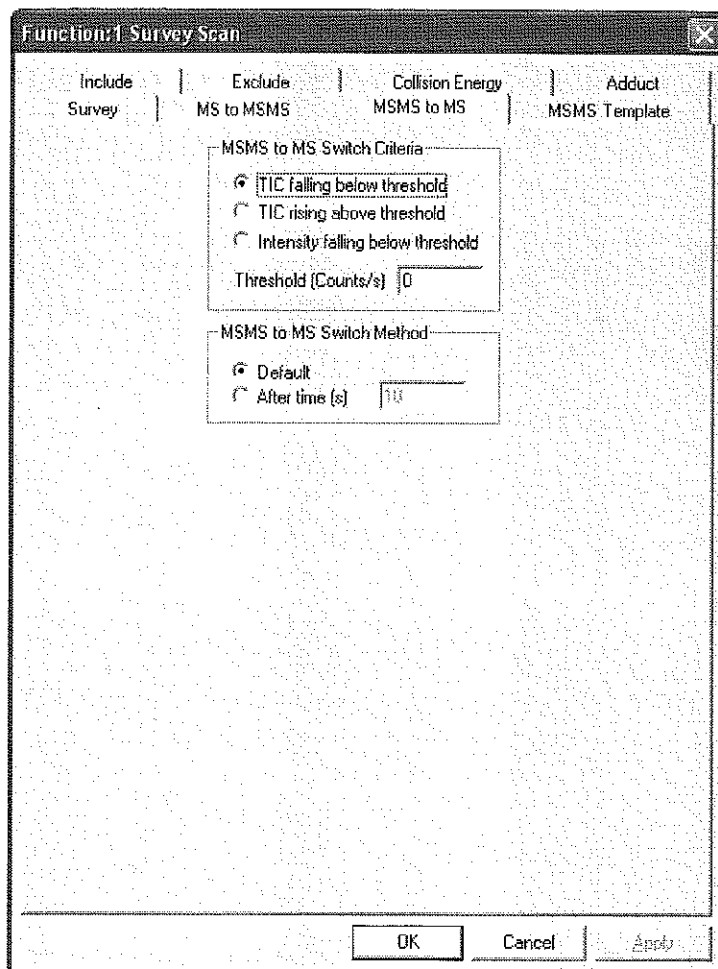


Figure 5-18 Survey Scan Function Editor: MSMS to MS Page

When MS/MS functions have been generated, they are carried out in parallel until the conditions for switching to MS are satisfied. When all MS/MS functions have stopped, the MS survey function is again carried out.

Table 5-12 describes the MSMS to MS page parameters.

Table 5-12 Survey Scan Function Editor: MSMS to MS Page Parameters

| Parameter | Description |
|-----------------------------------|--|
| MSMS to MS Switch Criteria | |
| TIC falling below threshold | When selected, the switch from MS/MS to MS scanning occurs when the TIC of the spectrum falls below the specified Threshold value. |
| TIC rising above threshold | When selected, the switch from MS/MS to MS scanning occurs when the TIC of the spectrum rises above the specified Threshold value. |
| Intensity falling below threshold | When selected, the switch from MS/MS to MS scanning occurs when the intensity of the largest peak falls below the specified Threshold value. |
| Threshold (Counts/s) | The value at which the switch from MS/MS to MS scanning occurs. |
| MSMS to MS Switch Method | |
| Default | When selected, the MS/MS function stops when the MS/MS to MS switch criteria are met. |
| After time (s) | When selected, the MS/MS function stops when the MS/MS to MS switch criteria are met, or otherwise when the specified time has elapsed. |

Including Masses

The Include page (Figure 5-19) allows masses (m/z) that are of interest, and prompt a switch to MS/MS operation, to be set-up.

The screenshot shows a dialog box titled "Function: 1 Survey Scan" with a close button (X) in the top right corner. The dialog is divided into several sections:

- Survey Include:** A tabbed section with "Survey Include" selected.
- MS to MSMS:** A section with "Exclude" selected.
- MSMS to MS:** A section with "Collision Energy" selected.
- MSMS Template:** A section with "Adduct" selected.
- Include Mass:** A section containing:
 - A checked checkbox for "Range" followed by an empty text input field.
 - A checked checkbox for "File" followed by an empty text input field and a "Browse..." button.
 - A table with columns: "Mass", "Retention Time", "Collision Energy", "Cone Voltage", "Charge State", and "Scan". The table is currently empty.
 - A horizontal scrollbar below the table.
 - Buttons: "New", "Add...", "Delete", "Save", and "Save As..."
- Include Window +/- (mDa):** A text input field containing the value "100".
- Charge State:** A section containing:
 - A checked checkbox for "Use Include By Charge State".
 - A text input field for "Charge State(s)" which is empty.
 - A text input field for "Number of Include Components" containing the value "10".
- Buttons:** "OK", "Cancel", and "Apply" buttons at the bottom right.

Figure 5-19 Survey Scan Function Editor: Include Page

These masses are listed in the Include List. It is normally used when the Survey Scan Function Editor MS to MSMS page Included Masses only option, or Included Masses Take Priority option is selected.

Table 5-13 describes the Include page parameters.

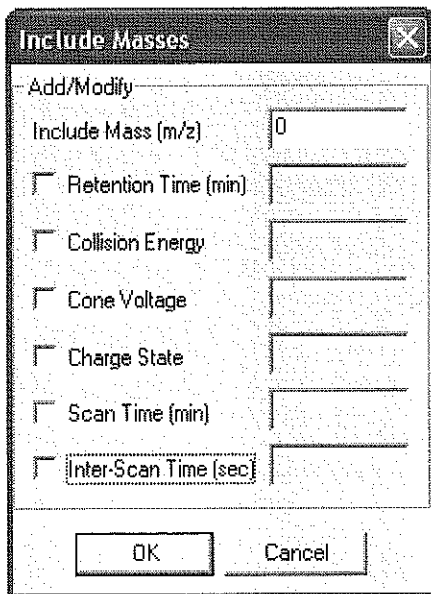
Table 5-13 Survey Scan Function Editor: Include Page Parameters

| Parameter | Description |
|------------------------------|---|
| Include Mass | |
| Range | Specifies the required masses (m/z), or range of masses, to be used from those in the Include List Box. (Separate individual masses by commas; use an underscore to denote a range of masses, e.g., 510, 520, 550_600, 700.) |
| File | When selected, the name of the file in which the Include List is to be stored can be entered in the adjacent text box; alternatively, use the Browse button to select an existing file. |
| New | Resets all the options and clears the Include List. |
| Add | Opens the Include Masses dialog box, which allows the Include List to be edited (see "Editing the Include List" on page 97). |
| Delete | Deletes the selected entry from the list. |
| Save | Saves the Include List details to an existing file. |
| Save As | Saves the Include List details to a new file. |
| Include Window +/- (mDa) | Defines the Include Window size, in mDa. If an interesting mass is detected from the survey scan, and is close enough (within the Include Window) to the specified include mass, and all other criteria are met, the mass will be switched on. If the mass is outside the Include Window, it will not be switched on. |
| Charge State | |
| Use Include By Charge State | Select to use charge states. |
| Charge State(s) | Enter the required charge state(s). |
| Number of Include Components | Enter the number of components to be included. |

Editing the Include List

1. On the Survey Scan Function Editor's Include page, click Add to open the Include Masses dialog box (Figure 5-20).

Note: To edit an existing entry in the Include List, double-click it.



The screenshot shows a dialog box titled "Include Masses". It has a standard Windows-style title bar with a close button (X). Below the title bar, there is a section labeled "Add/Modify". This section contains a list of parameters, each with a checkbox and a text box. The "Include Mass (m/z)" parameter is selected, and its text box contains the value "0". The other parameters are "Retention Time (min)", "Collision Energy", "Cone Voltage", "Charge State", "Scan Time (min)", and "Inter-Scan Time (sec)". At the bottom of the dialog are "OK" and "Cancel" buttons.

Figure 5-20 Include Masses Dialog Box

2. To enter a value, select the check box for the required option and enter the value in the adjacent text box.

Note: The text box becomes available only when you select an option.

Table 5-14 describes the Include Masses dialog box parameters.

Table 5-14 Include Masses Dialog Box Parameters

| Parameter | Description |
|-----------------------|--|
| Add/Modify | |
| Include Mass (m/z) | The specified mass (m/z) to include in the Function Switching process. |
| Retention Time (min) | If the specified mass elutes at the specified Retention Time and is within the MS to MSMS page Retention Time Window (s) value, the mass is considered to be on the Include List. This allows masses eluting from a column to be included as a mass of interest. |
| Collision Energy | The collision energy used to split the detected mass for the MS/MS scans. |
| Cone Voltage | The Cone Voltage value applied during the MS/MS scans. |
| Charge State | Include the mass by its charge state. |
| Scan Time (min) | The Scan Time, in minutes, used during MS/MS scans for the detected mass. |
| Inter-Scan Time (sec) | The Inter-Scan Time used during MS/MS scans for the detected mass. |

Excluding Masses

The Exclude page (Figure 5-19) is used to set-up masses that are to be ignored (i.e., not of interest) so that there is no switch to MS/MS operation. The Exclude List is a list of these masses.

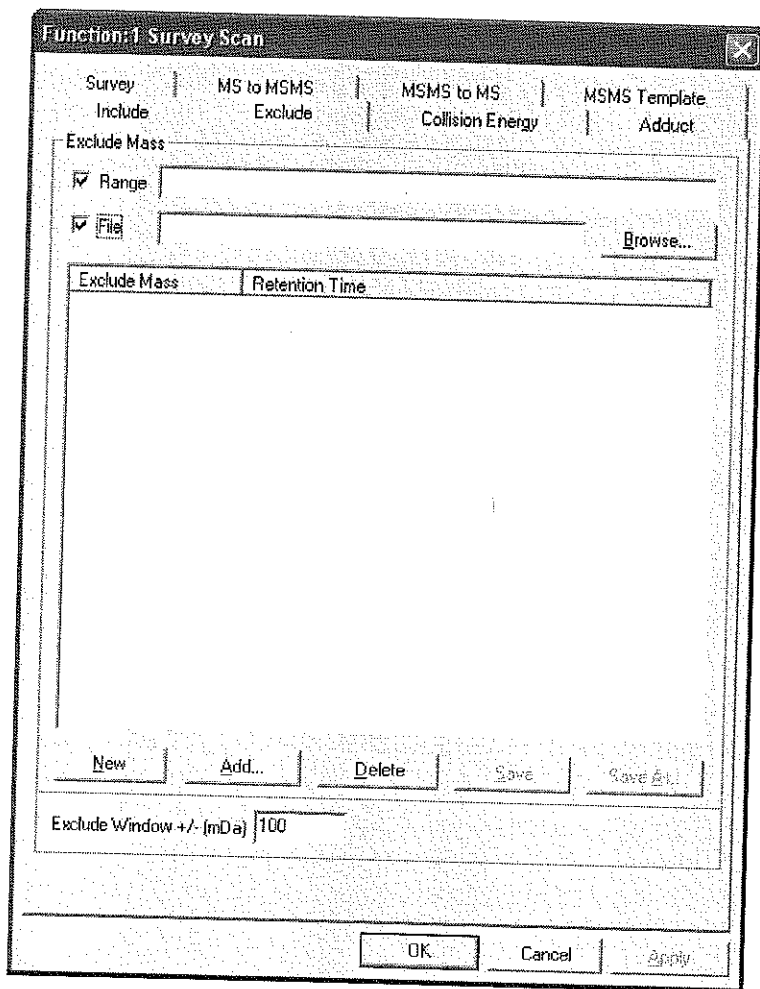


Figure 5-21 Survey Scan Function Editor: Exclude Page

Table 5-15 describes the Exclude page parameters.

Table 5-15 Survey Scan Function Editor: Exclude Page Parameters

| Parameter | Description |
|--------------------------|--|
| Exclude Mass | |
| Range | Specifies the required masses (m/z), or range of masses, to be used from those in the Exclude List. (Separate individual masses by commas; use an underscore to denote a range of masses, e.g., 510, 520, 550_600, 700.) |
| File | When selected, the name of the file in which the Exclude List is to be stored can be entered in the adjacent text box; alternatively, click the Browse button to select an existing file. |
| New | Resets all the options and clears the Exclude List. |
| Add | Opens the Exclude Mass dialog box, which allows the Exclude List to be edited (see "Editing the Exclude List" on page 101). |
| Delete | Deletes the selected entry from the list. |
| Save | Saves the Exclude List details to an existing file. |
| Save As | Saves the Exclude List details to a new file. |
| Exclude Window +/- (mDa) | Defines the Exclude Window size, in mDa. If a mass is detected from the survey scan, it is close enough (within the Exclude Window) to the specified exclude mass, and all other criteria are met, the mass will be ignored. |

Editing the Exclude List

1. On the Survey Scan Function Editor's Exclude page, click Add to open the Exclude Mass dialog box (Figure 5-22).

Note: To edit an existing entry in the Exclude List, double-click on it.

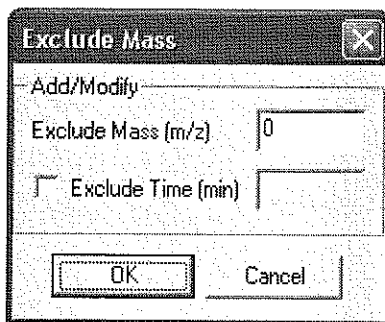


Figure 5-22 Exclude Mass Dialog Box

2. Enter the values as required.

Table 5-14 describes the parameters in the Exclude Mass dialog box.

Table 5-16 Exclude Mass Dialog Box Parameters

| Parameter | Description |
|--------------------|--|
| Add/Modify | |
| Exclude Mass (m/z) | The specified mass (m/z) to exclude from the Function Switching process. |
| Exclude Time (min) | If the Exclude Mass is detected at the Exclude Time, in minutes, and is within the MS to MSMS page Retention Time Window (s) value, it will be excluded from the Function Switching process. |

Selecting Collision Energy Options

The Collision Energy page (see Figure 5-19) allows you to set-up or select one of three Collision Energy options.

Function: 1 Survey Scan

Survey: MS to MSMS MSMS to MS MSMS Template
Include Exclude Collision Energy Adduct

Default Collision Energy

Use Default Collision Energy

Collision Energy Profile

Use Collision Energy Profile

File: _____ Browse...

Modify...

Charge State Recognition

Use Charge State Recognition

Maximum Number Of Charge States: 1

CS1 File: _____ Browse...

CS2 File: _____ Browse...

CS3 File: _____ Browse...

CS4 File: _____ Browse...

Modify...

OK Cancel Apply

Figure 5-23 Survey Scan Function Editor: Collision Energy Page

Table 5-15 describes the Collision Energy page parameters.

Table 5-17 Survey Scan Function Editor: Collision Energy Page Parameters

| Parameter | Description |
|------------------------------|--|
| Default Collision Energy | |
| Use Default Collision Energy | Uses a single default value, which is configured on the MSMS Template page (see Figure 5-16). This allows either the Tune window value or the value specified on the MSMS Template page to be used. If the Tune window value is to be used, the value can be changed during the scan, but it only becomes effective on the next switch from MS to MS/MS. |
| Collision Energy Profile | |
| Use Collision Energy Profile | <p>Selecting this option enables the controls in the Collision Energy Profile frame.</p> <p>The Collision Energy Profile is a range of Collision Energy values, which are associated with a specified mass. You can enter up to five different Collision Energy values.</p> <p>When an interesting mass is detected in an MS scan, and the mass is on the Collision Energy Profile list, these Collision Energy values are used during the MS/MS scans for the mass.</p> |
| File | The file name of the Collision Energy Profile file. |
| Browse | Allows you to browse for the required Collision Energy Profile file. |
| Modify | Opens the CE Profile dialog box, which allows a table of collision energy profiles to be created for masses that are being switched on (see "Creating a Table of Collision Energy Profiles" on page 105). |

Table 5-17 Survey Scan Function Editor: Collision Energy Page Parameters (Continued)

| Parameter | Description |
|---------------------------------|--|
| Charge State Recognition | <p data-bbox="498 322 1236 383">Selecting this option enables the controls in the Charge State Recognition frame.</p> <p data-bbox="498 404 1236 534">When a mass of interest is detected in an MS scan, its charge state is calculated. Using the mass and its charge state, a Collision Energy value can be obtained from the entered charge state table. This value is then used during MS/MS scanning.</p> <p data-bbox="498 555 1236 722">The Include page's Include by Charge State and the Collision Energy page's Use Charge State Recognition functionalities can be used independently, or both can be configured to combine their individual functionalities (see "Charge State Recognition Functionality" on page 108, for further details).</p> |
| Maximum Number Of Charge States | <p data-bbox="498 743 1215 843">Enter the number of charge states to be considered (maximum four) in this text box; the following CSx File text boxes are enabled, as appropriate, by this action.</p> |
| CS1 File, etc. | <p data-bbox="498 857 1215 921">Enter the file name of the required charge state file, or use the adjacent Browse button to search for a file.</p> |
| Modify | <p data-bbox="498 939 1243 1038">Opens the Modify Charge State dialog box; this is used to create, or modify charge state files (see "Creating or Modifying Charge State Files" on page 106, for details).</p> |

Creating a Table of Collision Energy Profiles

To create a Collision Energy Profile Table for masses that are being switched on:

1. On the Survey Scan Function Editor's Collision Energy page, select Use Collision Energy Profile; the associated Modify button is enabled.
2. Click Modify to open the CE Profile dialog box (Figure 5-24).

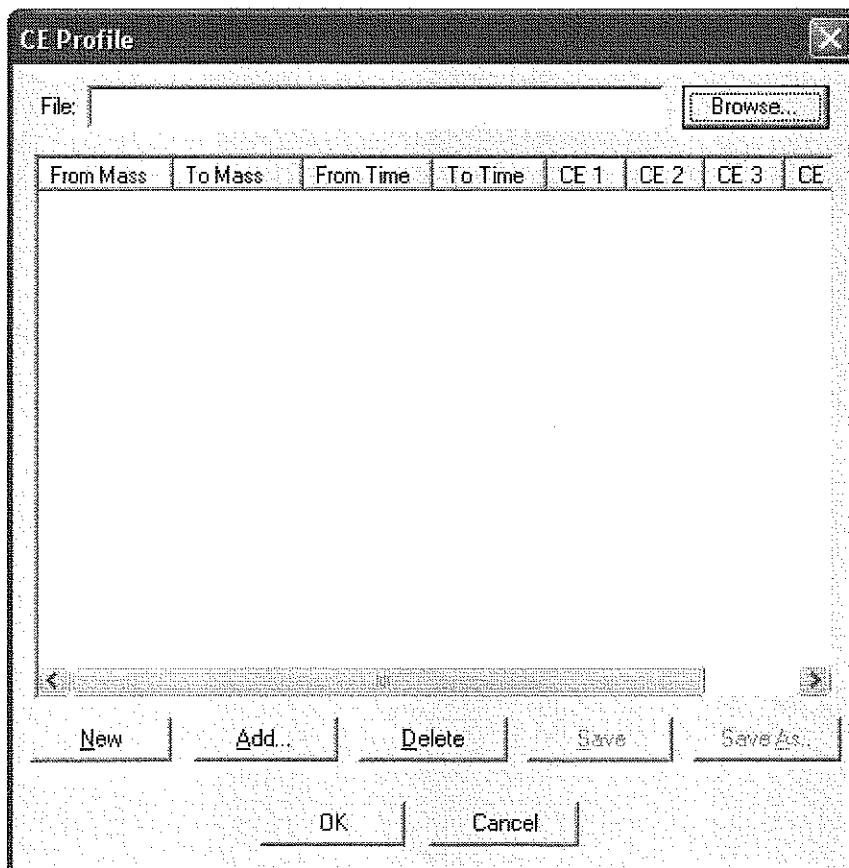


Figure 5-24 CE Profile Dialog Box

3. Click Add, or double-click an existing entry, to open the Collision Energy dialog box. This allows new entries to be added to the Collision Energy Profile Table. Table 5-18 describes the Collision Energy dialog box parameters.

Table 5-18 Collision Energy Dialog Box Parameters

| Parameter | Description |
|-----------------|---|
| Add/Modify | |
| From Mass (m/z) | Start mass (m/z) for the mass range in which the collision energy profile is to be used. |
| To Mass (m/z) | End mass (m/z) for the mass range in which the collision energy profile is to be used. |
| From Time (min) | Start time for the time range in which the collision energy profile is to be used. |
| To Time (min) | End time for the time range in which the collision energy profile is to be used. |
| CE1, etc. | The five allowed collision energy values, which will be applied to a relevant mass at a relevant time during MS/MS scanning. Selecting the check box for one value enables the check box for the following value. |
| OK | Closes the Collision Energy dialog box and enters the selected values in the CE Profile dialog box Collision Energy Profile Table. |

Creating or Modifying Charge State Files

Use the Modify Charge State dialog box (Figure 5-25) to create or modify charge state files.

1. On the Survey Scan Function Editor's Collision Energy page (see Figure 5-23), select Use Charge State Recognition.
2. Enter the required Maximum Number of Charge States.
3. Click Modify to open the Modify Charge State dialog box.

Note: The number of Modify CS tabs displayed in this dialog box corresponds with the Maximum Number of Charge States selected in step 2.

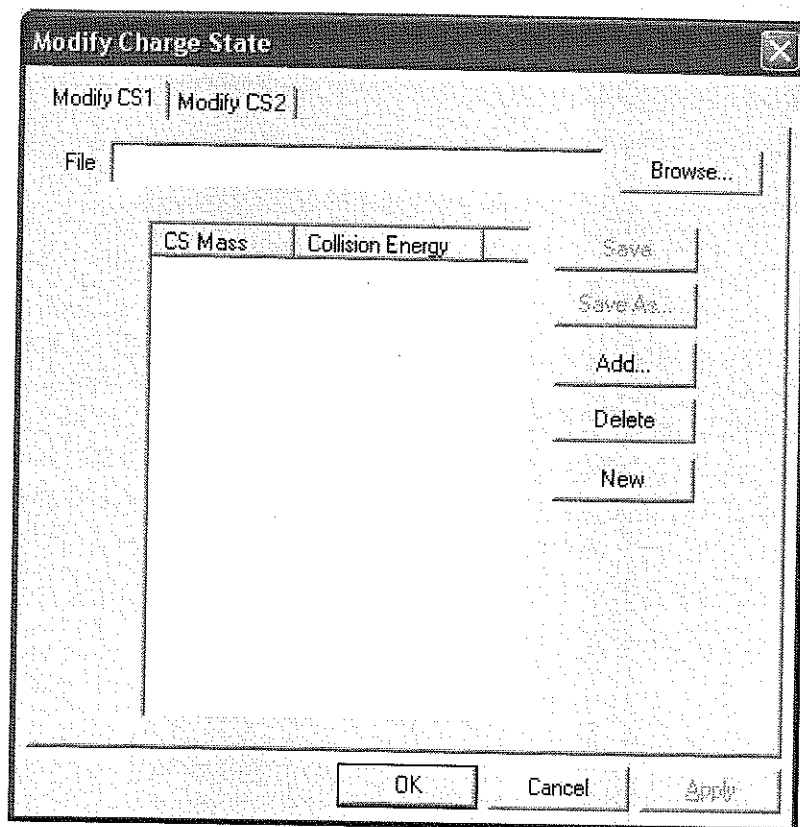


Figure 5-25 Modify Charge State Dialog Box

Adding New Entries to the Collision Energy Profile Table

1. Click the appropriate Modify CS tab.
2. Click Add or double-click an existing entry to open the Charge State Mass dialog box.
3. Enter the required values. Table 5-19 describes the parameters in the Charge State Mass dialog box.

Table 5-19 Charge State Mass Dialog Box Parameters

| Parameter | Description |
|------------------|---|
| Add/Modify | |
| CS Mass (m/z) | Specifies the mass (m/z) of interest. |
| Collision Energy | Sets the correct collision energy value to break up the mass of interest. |
| OK | Closes the Charge State Mass dialog box and enters the selected values in the Modify Charge State dialog box Mass List. |

Saving a Collision Energy Profile Table

Click Save to save the collision energy profile table to a text file. If the collision energy profile table has not been saved previously, a standard Windows dialog box is opened, allowing you to specify the file name and path. Click Save As to save an existing list to a new filename.

Opening a Collision Energy Profile Table

Click Browse to navigate to the collision energy profile table text file, or enter the filename and path in the File text box.

Charge State Recognition Functionality

You can apply charge state recognition functionalities from the Survey Scan Function Editor Include (see Figure 5-19) and Collision Energy (see Figure 5-23) pages. The functionalities, Include by Charge State and Use Charge State Recognition, can operate independently or together.

Using Charge State Recognition with Include by Charge State Disabled

This allows the software to switch on any peak of interest, but a collision energy value will be calculated only for those masses with a charge state that matches those set up in the Charge State Recognition frame. For all other masses, the default collision energy will be applied; this is either the Tune window value, or the value specified in the Survey Scan dialog box MSMS Template page.

For example, if the Include by Charge State option is disabled, and the Charge State Recognition has been configured for Charge States 1 and 2, any detected mass of interest switches on in the normal way. Masses switched on with a charge state of 1 or 2 will have

a collision energy value calculated from the Charge State Recognition table. All other masses use the specified default collision energy value.

Using Charge State Recognition with Include by Charge State Enabled

This restricts the masses that are switched on to those with the correct charge state. A collision energy value will be calculated for those masses with a charge state that is configured in both Include by Charge State and Charge State Recognition. For masses with a charge state which is only on the Include by Charge State list, a default collision energy value will be used.

For example, if the Include by Charge State section is configured to allow charge states of 2 and 3, and the Charge State Recognition section is configured for Charge States 1 and 2, only masses with a charge state of 2 or 3 are switched on. Masses switched on with a charge state of 2 will have a collision energy value calculated from the Charge State Recognition table. Masses with a charge state of 3 will use the specified default collision energy value.

Creating an Adduct List

In MassLynx, an adduct is the mass of an ion added to an original mass.

The Adduct page (Figure 5-26) allows you to create a list of such masses.

If a mass of interest (target mass) on the Include List is detected, the mass spectrometer is switched to MS/MS operation and that mass is added to the Exclude List, so that it is ignored if detected again. Also, when the target mass is detected, any mass in the Adduct page adduct list is added to that target mass, and the resulting mass added to the Exclude List so that the adduct is also ignored if it is detected.

For example, for positive ion operation:

If the molecular target mass (M) is 200, the target mass (m/z) on the Include List is 201 ($M + H^+$). When detected, a switch to MS/MS operation is triggered and the mass is added to the Exclude List.

A typical adduct is sodium (Na , mass 23), for which the target adduct mass is 223 ($M + Na^+$). Hence the value to be added to the Adduct page adduct list is $(M + Na^+) - (M + H^+) = (223 - 201) = 22$.

Similarly, if the adduct is ammonium (NH_4 , mass 18), the target adduct mass is 218 ($M + NH_4^+$). Hence the value to be added to the Adduct page adduct list is $(M + NH_4^+) - (M + H^+) = (218 - 201) = 17$.

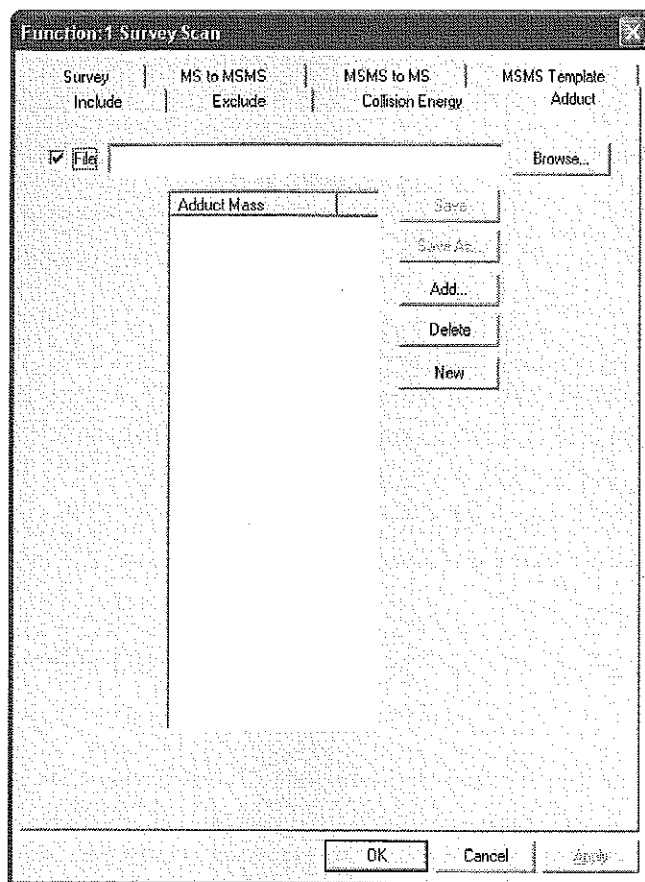


Figure 5-26 Survey Scan Function Editor: Adduct Page

Adding an Adduct Mass to the Adduct List

1. Click Add, or double-click an existing mass in the adduct list to open the Adduct Mass dialog box.
2. Enter the required value in the Add/Modify, Step Size (m/z) text box.
3. Click OK to add the value to the adduct list.

Saving an Adduct List

Click Save to save the adduct list to a text file. If the adduct list has not been saved previously, a standard Windows dialog box is opened, allowing you to specify the file name and path. Click Save As to save an existing list to a new filename.

Opening an Existing Adduct List

Click Browse to navigate to the adduct list text file, or enter the filename and path in the File text box.

5.7.7 Monitoring Acquisitions

When an acquisition is started, the Function Switching Status dialog box opens, showing the precursors currently running.